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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Lal *et al.*

Application No. 09/763,397

Filed: February 16, 2001

For: RECOMBINANT MULTIVALENT MALARIAL VACCINE AGAINST PLASMODIUM FALCIPARUM

Examiner: Vanessa L. Ford

Date: December 20, 2002

Art Unit: 1645

CERTIFICATE OF MAILING

I hereby certify that this paper and the documents referred to as being attached or enclosed herewith are being deposited with the United States Postal Service on _____ as First Class Mail in an envelope addressed to: COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231.

_____
William D. Noonan, M.D.
Attorney for Applicant

COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SECOND DECLARATION UNDER 37 C.F.R. § 1.131

I, Ya Ping Shi, hereby declare as follows:

1. I am a co-inventor of the subject matter described and claimed by the patent application referenced above, *i.e.,* United States application No. 09/763,397 (hereafter the '397 application). I currently am employed by the Centers for Disease Control and Prevention (CDC), the assignee of the '397 application, which is located in Atlanta, Georgia. I was employed by the CDC while developing the invention described and claimed in the referenced application.

2. I understand that claims pending in the present application have been rejected in view of Tine *et al.*, *Infection and Immunity*, 64(9): 3833-3844, 1996. I understand that Tine *et al.* has been cited as allegedly anticipating certain claims pending in the referenced application, or, in the alternative, as allegedly rendering the claimed embodiments obvious.

3. The publication date of Tine *et al.* is September 1996. United States Provisional Application No. 60/097,703 was filed on August 21, 1998. However, the co-inventors named on the '397 application invented the subject matter covered by the claims pending in the '397 application prior to the September 1996 date that Tine *et al.* became available as a reference.

4. I previously executed a first Declaration under 37 C.F.R. § 131, including the attached Exhibits A and B, in connection with Applicant's June 11, 2002 amendment and response. Exhibit A consists of true and accurate facsimile photocopies of 21 corresponding pages from my laboratory research notebook. Exhibit B consists of one page of CDC Biotechnology Core Facility Records, showing my request for oligonucleotide synthesis, and the sequences of the requested oligonucleotides. This request was made prior to September 1996. These oligonucleotides were used in the reduction to practice of the invention, as described in Applicant's June 11, 2002 amendment and response. The contents of these pages of Exhibits A and B, and pertinent statements made on these pages are discussed in detail in Applicant's June 11, 2002 amendment and response.

5. Exhibits A and B were previously submitted as evidence that the conception and reduction to practice of the invention recited in the claims of the '397 application occurred in the United States of America prior to November 1997, the effective date of the Gilbert *et al.* publication cited as allegedly anticipating prior art in the Office action mailed February 11, 2002. As noted on my previous Declaration, all dates stated on Exhibits A and B were redacted prior to submission, but were made prior to November 1997, the effective date of the Gilbert *et al.* publication.

6. Similarly, all dates stated on Exhibits A and B were prior to September 1996, the effective date of the Tine *et al.* publication.

7. All statements made herein and of my own knowledge are true and all statements made on information are believed to be true. Furthermore, these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements made may jeopardize the validity of the application or any patent issuing thereon.

_____ _____

Ya Ping Shi, Ph.D. Date

EXHIBIT A

First PCR

	Tm		
AA: G0 - G2	52.6	94°C 5min	
BB. G3 - G6	56.56	94°C 45"	
CC. G7 - G12	61.61	45°C 1min	8 cycle (p139)
		72°C 1.5min	

16ul dNTP
10ul Buffer
0.5ul Enzy
26.5ul.

50ng/each.

73.5

AA: 2 × 4 = 8ul 65.5

BB 2 × 4 = 8ul 65.5

CC. 2 × 6 = 12ul 61.5

Second PCR.

53.5

16ul dNTP
10ul buffer
0.5ul Enzy
5ul Oligo 1
5ul Oligo 2
46.5

94°C 5min.
94°C 45"
45°C 1min
72°C 1.5min
} 25 cycle (p141)

AA	DD1	1ul	52.5+5+5	
	DD2	2.5ul	51 +5+5 →	G0
	DD3	5ul	48.5+5+5	G2 ✓
	DD4	10ul	43.5 +5+5	
BB	EE1	1ul	52.5+5	
	EE2	2.5ul	51 + 5	G3 ✓
	EE3	5ul	48.5+5 →	G6 ✓
	EE4	10ul	43.5+5	
C	FF1	1ul	52.5 +5	
	FF2	2.5ul	51+5 →	G7 ✓
	FF3	5ul	48.5+5	G12
	FF4	10ul	43.5+5	

Redo CC_0: G7 - G12 = 12ul.
dNTP 16ul c; Taq.
10x Buffer 10ul.
H_2O 61.5ul

100ul

94°C 5min
94°C 45"
40°C 1min
72°C 2min
) 8 cycle.

CC_1' G7 - G8 (only do second PCR) = 4ul + 69.5ul H_2O
CC_2' ✓ G9 - G12 2×4 = 8ul + H_2O . 65.5 —

Do SOE G0 - G76

	$DD_1 + EE_1$	H_2O	63.5		
GG_1	1ul + 1ul = 2ul	61.5		16ul	dNTP.
GG_2	2.5ul + 2.5ul = 5ul	58.5		10ul	Buffer.
GG_3	5ul + 5ul = 10ul	53.5		5ul	G0
GG_4	10ul + 10ul = 20ul	43.4		5ul	G76
				0.5	Taq
					36.5

program 141

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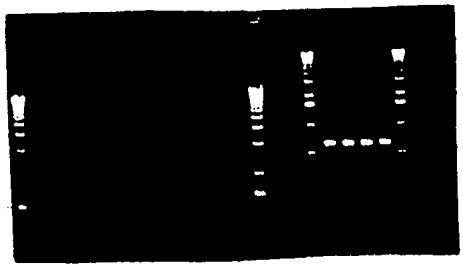
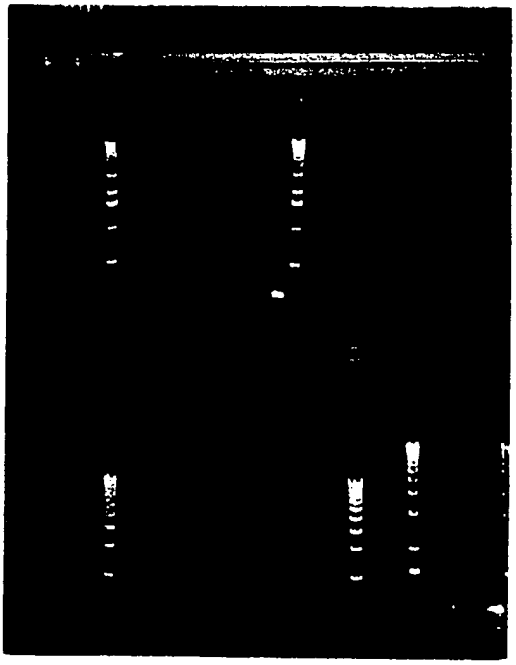
		primers	63.5	H_2O	
FF_1	CC_1'		1ul	62.5	16ul dNTP
FF_2		G7) 10ul	2.5ul	61	10ul Buffer
FF_3		G12	5ul	58.5	oligos 10ul.
FF_4			10ul	53.5	Taq 0.5
					36.5
FF_5	CC_2'	primers	1ul		94°C 5min
FF_6			2.5ul		94°C 45"
FF_7		G9) 10ul	5ul.		40°C 1min
FF_8		G12	10ul		72°C 2min

5 cycles #41

Result: G$_{G_1}$-4





F$_{1-3}$ did not work probably because oligo?

prepare new temp oligo G$_4$ - G$_{12}$. also AL-106S.

Redo: $CC_2' \rightarrow CC_2''$ and CC_3''.

CC_2'' G9 G10 G11 G12 × 2 = 8ul · 65.5 (73.5)

CC_3'' G9 G10 G11 AL1064 × 2 = 8ul · 65.5

× works well

16ul dNTP ·
10ul Buffer ·
0.5 Taq

Same to before.

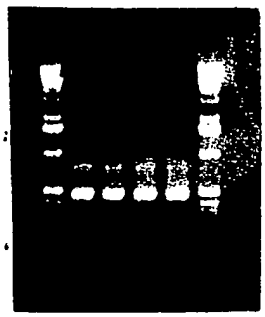
Second PCR.

					H2O
FF_1''	} CC_2''	primers		1ul	62.5
FF_2''		G9		2.5ul	61
FF_3''		G12		5ul	58.5
FF_4''				10ul	53.5

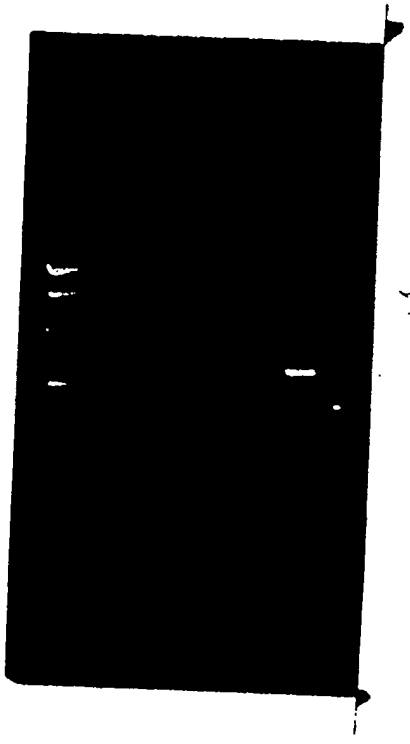
16ul dNTP
10ul buffer
Oligo 10ul
Taq 0.5ul.

FF_5'', FF_6'', FF_7'', FF_8'' } CC_3'' G9 AL1064 works well

Same to before.
1141



II4



II

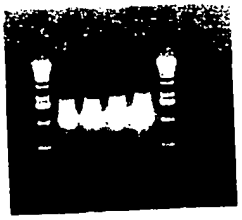
SOE for G7 - G7 + AL-1065

	CC1	FF5	H2O	
HH1	1 ul	+ 1ul	61.5	16ul dNTP
HH2	2.5ul	+ 2.5ul	58.5	10ul buffer
HH3	5ul	+ 5ul	53.5	5ul G7
HH4	10ul	+ 10ul	43.5	5ul AL-1065
			65.5	0.5 Taq
				365

program #41

	GG7	+ HH1,	H2O	63.5	16ul dNT
II1	1ul	+ 1ul	61.5		10ul buffer
II2	2.5ul	+ 2.5ul	58.5		5ul AL1064
II3	5ul	+ 5ul	53.5		5ul AL1065
II4	10ul	+ 10ul	43.5		0.5 Taq
					365

program #41



good!

Further cleaning and cloning.

Sequencing.

A: Run gel and cut and clean.

1) gene clean (from product of PCR)

2) gel clean through column (according instruction of manufacturer) (50ul of PCR product tube one is pellet (store in −20°c)

another has 20ul water. From this, 10ul was take for digestion.

B: digestion:

Not I : 26ul water.
3ul Buffer
1ul Not I

1h 37°c

(II, Gene clean
II, column clean)

pellet.

↓

BamH I 26ul H2O
3ul buffer
1ul BamHI

1h 37°c

Ligation.

			control I	control II
Water	13ul		"	15ul
Vector	1ul	(BamHI and Not I digest)	"	
5x lig buffer	4ul.		"	
T4 ligase	2ul		"	x

over night (4°c

0

Not I digestion:

Vector:

1ml	Vector (linear 3.2ug/ml)
3ul	10 x buffer
3ul	BSA
4 ul	Not I
10 ul	H2O

30ul 37°C 1.5h

target

II 2 and control (MSp-1)

23ul	H2O
3ul	BSA
3ul	10 x Buffer
1ul	Enzyme

30ul 37°C 1.5h

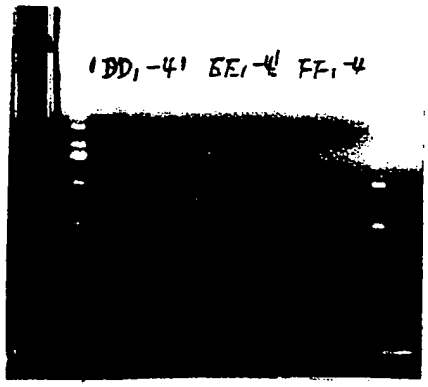
BamHI digestion

Vector

BamHI	4ul
Buffer	3ul
water	23
	30ul 37°C 1.5h

[illegible]	2ul
Buffer	3ul
water	25ul
	30ul 37°C 1.5h

Result



FF4-4 did not work because first PCR (CC) annealing temp was too high

Need redo CC (first PCR), then FF1-FF4

ligation as before
transformation as before

result. not so much white clones. probably vector was not properly digested.
Change further purify vector.

pick up 40 clone grow overnight.
cell PCR: as regular. 10ul cell ~94°c 5min.

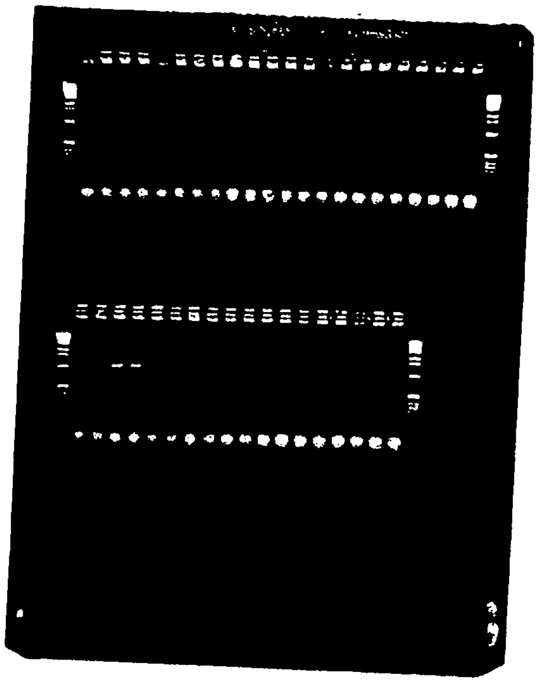
oligo	AL1064	2.5ul
	AL1065	2.5ul
	Buffer	5ul.
	dNTP	8ul.
	Taq	0.5
	H2O	21.75
		40ul.

15 cycle 94.°c 45" 55°c 45" 72°c 2m

positive clone.

1, 2, 3, 4, 6, 8, 17, 21, 22,
25, 26, 27, 31, 33, 36, 39, 40,

Grow back



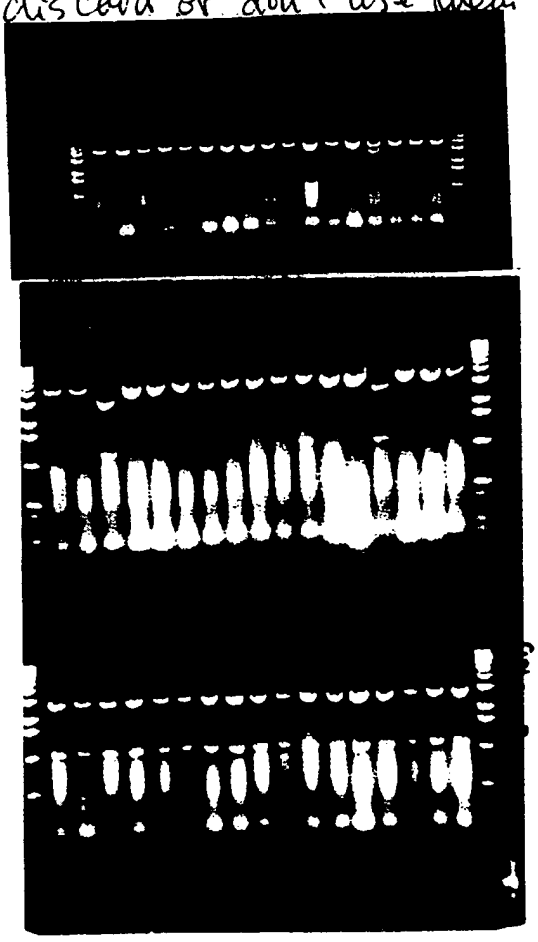
Save gel

Saulas SGV/R = Synthetic gene Vaccine
Darden design

digest all positive (17) clones (based on pcR)

Single digestion : BamHI or NotI
double digestion BamHI and NotI.

Result: Clone 3. 26. 33 are not pure clones.
discard or don't use them



2/6

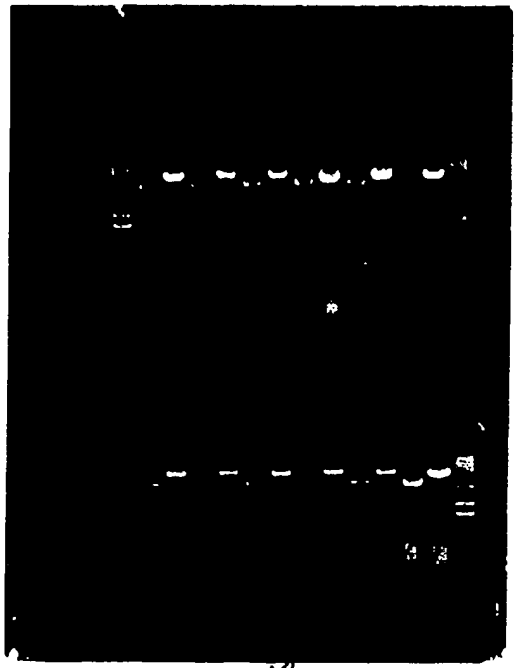
Plasmid pBacPAK8 and pBacPAK9 (from Sajor
2ng/1ocul 2ng/1ocul

Transformation:
1ocul plasmide (200ng)
1ocul XL-blue cell

procedure as regular.

plating: overnight
growth well

Miniprep of pBacPAK8 and pBacPAK9 -
run undigested and digested plasmid



100ng/ul ×19 =
1a 9ul.

13

This result confirm that. ~~xx~~ orders are no problem. also confirm (11) (20) (63) clones are true clones:

Qwill sequence clone 20.

Methylation:

Clone 63 Vector correct.
Clone 20 Nest target correct

Clone 63 methylation.

Fixation:	3ul	TaqI methylation
	3ul	NEB 4 Buffer
	0.3ul	BSA
	22.2ul	1+20
	1.5ul	1Mix SAM

1 hr 65°C

Mix: 50ul NEB4 Buffer + 450ul 1+20 + 1.25ul SAM

0.6ul NaCl (5M)
60ul Ethanol (100%)

HInd II cut clone 63 (two piece vector bj
clone 20 (more intense vector small

Run gel Standard 20 63 Standard
(more small (+ve bj)

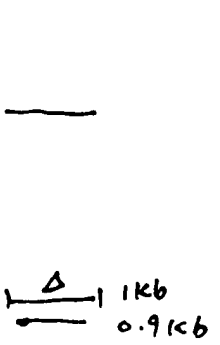
Digestion condition:

3 ul	buffer
6 ul	HindIII
21 ul	H_2O

1.5h 37°C

Result:

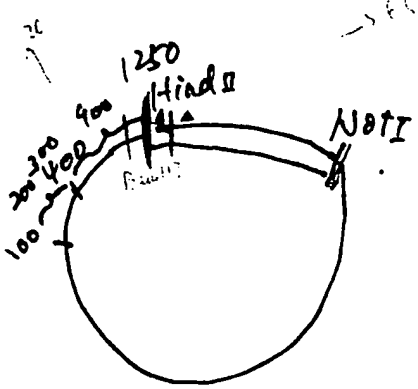
clone 20



— 0.2 kb

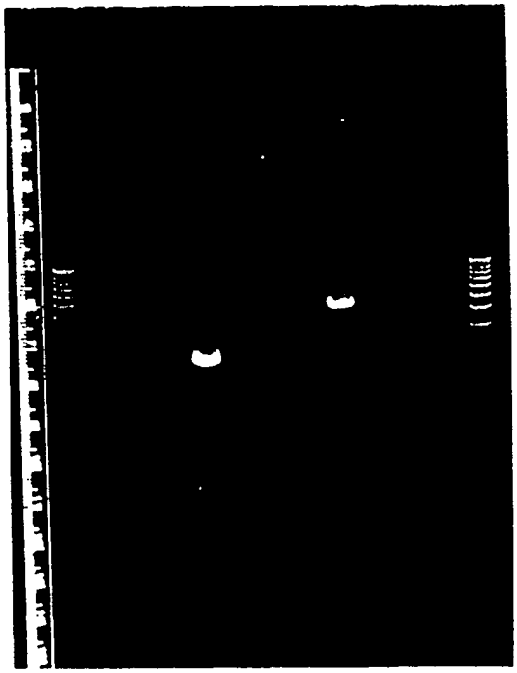
clone 63

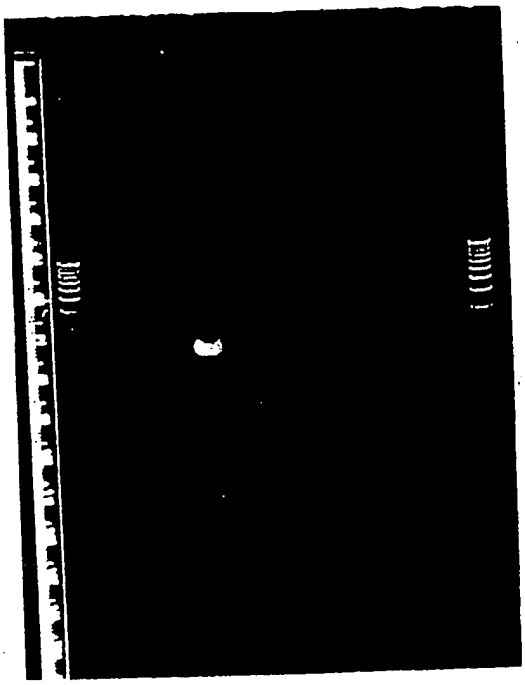
— — 1Kb



△ cut fragment

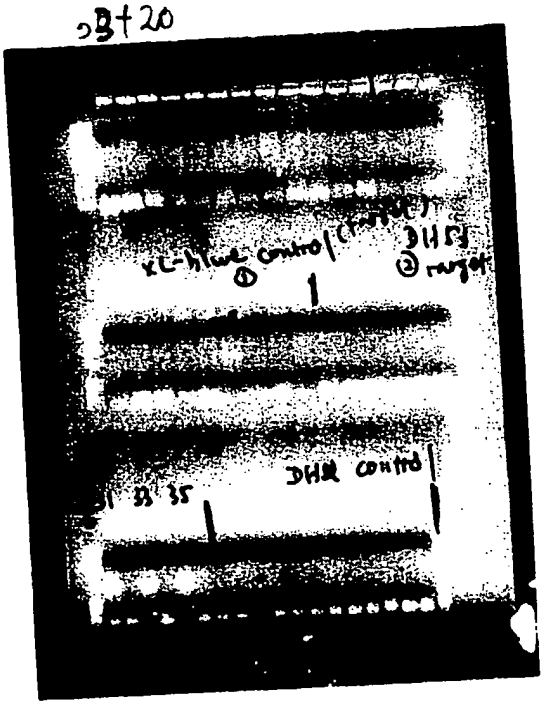
ligation: as routine





Clone 63 + 20 ligation see before

Clone PCR Primer: AL1097
AL1064



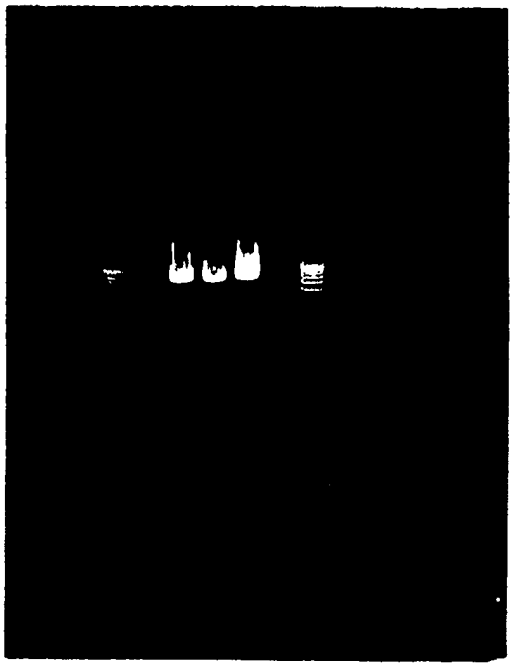
clones 21, 31, 33, 35 are positive.

save as name:

~~38cIS/cL-D121/63+20~~

Pac8/63+20/number

BamHI digestion: 63+20 (21, 31, 33, 35) 6



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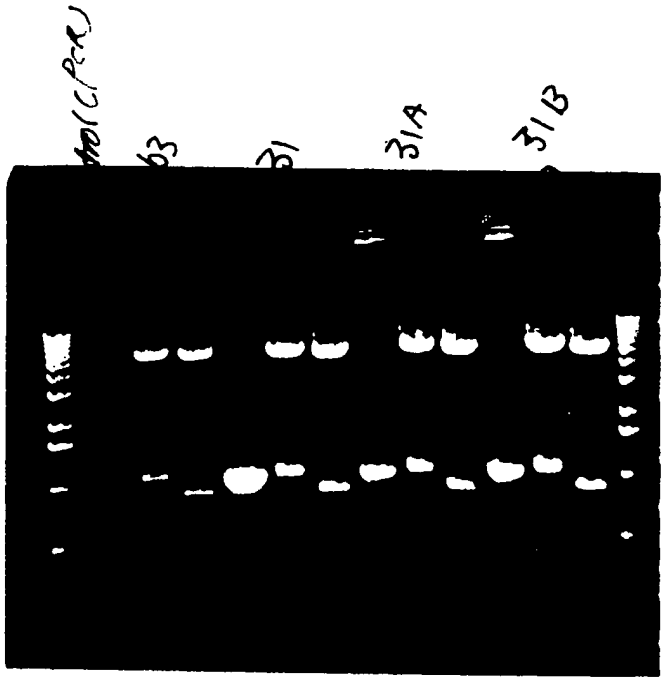

Save clone 31A and 31B

named as pac8/63+20/31A and 31B

Date	Oligo ID	DNA Syn No.	Sequence	Length-mer
	G0	953882	GTCGGGATCCATGAAATTCTTAGTCAACGTTGCCCTTGTT TTTATGGTCGTGTACATTTCTTACATCTATGCGGatcatcatca tcatcatcat	94
	GL	953883	ttatcttcatctttaccttcatgatgatgatgatgatgat	40
	G1	953884	gaaggtaaagatgaagataaaAGAGATGGAAATAACGAAGACAAC GAGAAATTAAGGAAACCAAAACATAAAAAATTAAAGCAAC CAGGGGATGGTAATCcttggtccccatgtagtgta	120
	G2	953885	tatttacgacattaaacacaCTGGAACATTTTTCCATTTTACAAATTT TTTTTTCAATATCATTTTCATAATCTAATTCGTCTTTAGGTT TTCCACAAGTtacactacatggggaccaag	120
	G3	953886	TGTGTTTAATGTCGTAAATAGTAATTCTGGATGTTTCAGA CATTTAGATGAAAGAGAAGAATGTAAATGTTTATTAGAAG ATTCAGGTAGCAACGGAAAGAAAATCACATGTGAATGTAC	120
	G4	953887	tggtatatcttcacaatttCCATCAGGATTTGCATTGGGGTTTGCGT TTGGGTTTGCATTGAAATTGTCATATTGCACAATAGGCTT AGAATCAGGTTTAgtacattcacatgtgatttt	120
	G5	953888	gaaattgtgaagatataccaCATGTAAATGAATTTTCAGCAATTGAT CTTGGAAATGCTGAAAAATATGATAAAATGGATGAACCAC AACATTATGGGAAatcactcactccattagaag	120
	G6	953889	gcattgtttatgaatgtgttGCTTAGTTCAGTTATACCTATAAATTTAG AATTTGCTTTTATATACTGATACAAACTTTTATCATTTGGTT TATATAATTcttctaatggagtgagtgat	120
	G7	953890	aacacattcataaacaatgcTGGACAACATGGACATATGCATGGTA ACGAGAGGGAAGATGAGAGAACGCTTACTAAGGAATATG AAGATATTGTTTTGAaagagtttacatatatgata	120
	G8	953891	ctttaatttttcataatctGTTAAATGTTGTTCATATTGTTTAGGTTGA TCACTTTTTTGATATGGATGTTCCCAATAATTCTGTCCTCT TCCAAAGTTtatcatatatgtaaactctt	120
	G9	953892	agattatgaaaaaattaaagAAGGTAAGCCCTTGGATAAATTTGGA AATATCTATGATTATCACTATGAGCATTCTAGTCCATCTAG TACAAAGTCATCAagtccatcaaatgtaaaatc	120
	G10	953893	atccttatatttcgctaaaaCCTTTTCATAATAACTTATACCGAAGAA ATCTCTGATTTCAGCTTTAAATTTTTTCATTAATCTTGTAG CTAGACTagctgattttacatttgatggact	120
	G11	953894	ttttagcgaaatataaggaTGATTTAGAAGTTAGTATCAGGAGATA TGTTCGCAAGAATCAAAATAGATATAACTATTTTCTTAttgaa taggcggccgccgac	104
	G12	953895	gtcggcggccgcctattcaa	20

EXHIBIT B